REMARKS

The Official Action as well as the numerous formal rejections under '112 have again been carefully reviewed. The review indicates that the claims, especially as amended, recite patentable subject matter and should be allowed. Reconsideration and allowance are therefore respectfully requested.

The invention is directed to a method that results in an increase in the specific activity of a mutated glycosyl hydrolase on a substrate relative to an unmutated form of the glycosyl hydrolase, by replacing an active site associated glycosyl-stabilizing amino acid of the hydrolase with an amino acid binding cellobiose less tightly than the glycosyl-stabilizing amino acid, and further provides glycosyl hydrolase variants and mutants of Y245G, Y42R, or W82R – all of which constitute cellulase enzymes characterized by improvement over the wild-type enzyme in the catalytic digestion of cellulose substrates. The increased catalytic activity is provided for soluble and insoluble substrates.

The increased ability to solubilize cellulose, relative to their wild type counterparts, for instance, is for a number of glycohydrolases belonging to structural family 5 that have been identified as being structurally analogous to E1 and as having specific residues, the aromatic side chains of which may perform functions equivalent to that of Tyr-245 in E1 (Table 1 of specification, left column). Mutation of these residues to the residues listed in corresponding rows of the middle column (Trp39 of 1A3H; Trp171 of 1BQC; Trp212 of 1CEN; Phe229 and/or Phe258 of 1CZ1; Trp259 and/or Trp811 of 1EDG; Trp30 of 2MAN) are in accord with the computer modeling studies, to produce a decrease in the degree of product inhibition exhibited by the resulting mutant enzymes, relative to that exhibited by the wild-type enzymes, and as a result exhibit improved performance in the hydrolysis of cellulose.

Similarly, replacement of the residues listed in the right-hand column of Table I of the specification with residues having much less ability to form hydrogen bonds to the oxygen or hydrogen atoms of substrate hydroxyl groups are available to reduce the affinity of the enzyme active site for cellobiose. The mutant enzymes produceable in accordance with the invention are exemplified by the examples and Table 1.

Claim 7 which depended from claim 3 was rejected under the second paragraph of 35 USC 112 on allegations of indefiniteness; however, in view of the fact that claim 7 now recites the specific amino acid sequences, the rejection is no longer applicable.

Claims 7 and 31 were rejected under the second paragraph of 35 USC 112, also on allegations of indefiniteness; however, in view of the fact that these claims have been amended to eliminate the short oligonucleotide sequences in favor of the specific amino acids, this rejection is no longer application.

Claims 29 and 31 were rejected under the second paragraph of 35 USC §112 on allegations of indefiniteness; however, in view of the amendments made to these claims, it should now be clear to a person skilled in the art, which specific amino acid in the full length sequence of the enzyme is targeted for substitution – without undue experimentation.

Claims 3, 29 and 31 were rejected under the first paragraph of 35 USC §112 on allegations that the specification is not enabling for a method of increasing the specific activity of the E1 endoglucanase; however, in view of the amendments made to these claims and the fact that the claims should be read with reference to the specification commencing at page 6, line 16 through page 7, line 16, together with the results in Tables 1-4 (which make abundantly clear, the method of increasing the specific activity of the E1 endoglucanase comprising the mentioned amino acid sequences), the rejection is no longer applicable.

Claim 3 was rejected under the first paragraph of 35 USC §112 on allegations that the subject matter was not described in the specification in a manner such as to reasonably convey to one skilled in the art that applicants had possession of the claimed invention; however, applicants would retort that claim 3, as presently amended, in fact recites the method of enhancing a specific activity of any glycosyl hydrolase because – as mentioned on page 2 of the specification between lines 15 and 29, in these cellulase enzymes there are 21 families of catalytic domains, each classified on the basis of similarity of their amino acid sequences and the location where glycine may be substituted by tryptophan, or where glycine, alanine, valine, serine, etc. may be substituted (page 7, lines 1-5 – as well as Table 1) and page 7, lines 13-16 (where it is stated that 3 or 4 mutations were made for each E1 site that included Ala, Gly, Glu and Arg), as a process for making and using these enzymes using various mutagenesis kits for site directed mutagenesis (SDM) is unquestionably clear.

In view of the fact that no references have been cited in anticipation or obviousness of the invention—particularly, as currently recited in the amended claims, it is believed that the application is now in condition for allowance, and early notification of the same is earnestly solicited.

Respectfully submitted,

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